# Clinical Activity of Folinic Acid in Patients with Chronic Fatigue Syndrome

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## Summary

**A high incidence of severe B-cell immunodeficiency and chronic reactivated Epstein-Barr virus (EBV) infection in patients with chronic fatigue syndrome (CFS) is reported herein. Of the 58 patients evaluated, 100 % had evidence of prior EBV exposure and 72 % had evidence for reactivated EBV infection. Notably, 94 % of CFS patients had B-cell immunodeficiency with a marked depletion of their $CD19^+IgM^+$ mature B-lymphocyte population. A remarkable 81 % of CFS patients experienced subjective improvement of their symptoms after treatment with folinic acid (CAS 58-05-9, leucovorin). The findings provide unprecedented evidence that CFS frequently is a folinic acid responsive clinical entity accompanied by B-cell immunodeficiency and inappropriate antibody responses to EBV.**

## Zusammenfassung

**Klinische Wirkung von Folinsäure bei Patienten mit chronischem Müdigkeitssyndrom**

**Es wird über eine hohe Inzidenz schwerer Immuninsuffizienz der B-Zell-Reihe sowie chronischer Infektion mit reaktivertem Epstein-Barr-Virus (EBV) bei Patienten mit chronischem Müdigkeitssyndrom (chronic fatigue syndrome, CFS) berichtet. Von den 58 untersuchten Patienten waren 100 % früher mit EBV infiziert, und bei 72 % lag ein Labornachweis für eine reaktivierte EBV-Infektion vor. 94 % der CFS-Patienten wiesen eine B-Zellimmuninsuffizienz auf mit deutlich erniedrigten $CD19^+IgM^+$ reifen B-Lymphozyten. Bei 81 % der Patienten führte die Behandlung mit Folinsäure (CAS 58-05-9, Leucovorin) zu einer subjektiven Verbesserung ihrer Symptome. Diese Befunde deuten darauf hin, daß es sich beim CFS um ein auf Folinsäure ansprechendes klinisches Bild handelt, bei dem B-Zell-Immuninsuffizienz und mangelhaftes Antikörperansprechen auf EBV häufig vorkommen.**

## Key words

- CAS 58-05-9
- Chronic fatigue syndrome
- Epstein-Barr virus
- Folinic acid
- Immunodeficiency
- Immunomodulators
- Leucovorin

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## 1. Introduction

Chronic fatigue syndrome (CFS) as defined by the diagnostic criteria developed by the US Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA) has a prevalence of 0.1 %–0.3 % in the United States and affects mostly women between 25–45 years of age [1–10]. Patients with CFS have clinically evaluated, unexplained, persistent or relapsing fatigue plus four or more specifically defined associated symptoms [1–10]. There is a considerable overlap between the presenting features of CFS and fibromyalgia [7, 8, 11]. The pathogenesis of CFS has not yet been deciphered and no treatments have been reported to effectively alleviate the frequently debilitating fatigue and postexertional malaise of the affected patients [12–15]. Reports of clusters and outbreaks of CFS, including the 1934 LA County Hospital outbreak, 1955 London Royal Free Hospital outbreak, and 1985 Nevada Incline Village outbreak, prompted speculations of a potential infectious cause [16–22]. Epstein-Barr virus (EBV) was proposed in the mid-1980s as a likely etiologic viral agent for CFS; however, later observations in small series of patients raised questions about the accuracy of the proposed causal relationship between EBV infection and CFS [23–25].

Immunoglobulin M (IgM) and G (IgG) antibodies directed against the Epstein-Barr viral capsid antigen (VCA) are usually present at the onset of clinical illness during an acute EBV infection because of the long viral incubation period. Since the IgM VCA antibodies wane within 3 months, their presence is highly suggestive of an acute EBV infection. By comparison, IgG antibodies to early antigen (EA) are present at the onset of clinical illness and serve as a marker for acute new or reactivated EBV infection. In contrast, the IgG VCA antibodies persist for life and serve as a marker of prior EBV infection. IgG antibodies to EBV nuclear antigen (EBNA) appear 6 to 12 weeks after the onset of symptoms and persist throughout life; their presence early in the course of illness excludes acute infection. On the other hand, the appearance of EBNA antibodies in a patient who was previously VCA positive and EBNA negative is highly suggestive of recent EBV infection. Thus, while the presence of IgM VCA antibodies suggests the likely presence of acute EBV infection, the diagnosis is most certain in the presence of IgG and IgM VCA and the absence of IgG EBNA antibodies. EBV infections were associated with CFS but the cause of EBV infections in this patient population has not been studied. CFS patients were found to have inappropriate antibody responses to EBV suggestive of functional defects in their immune system [23–25].

Recent studies indicate that CFS is a central nervous system fatigue disorder [26]. CFS mimicks in many aspects the fatigue experienced by patients with cancer or inflammatory disorders treated with anti-folates (co-trimoxazole, methotrexate, pemetrexed). The folic acid analogues methotrexate used for treatment of cancer (such as acute lymphoblastic leukemia) as well as inflammatory disorders (such as rheumatoid arthritis) and pemetrexed used for treatment of cancer (such as non small cell lung cancer and mesethelioma) which bind to dihydrofolate reductase and prevent the synthesis of tetrahydrofolate as well as co-trimoxazole which causes sequential inhibition of folic acid synthesis by sulfamethoxazole and then by trimethoprim cause fatigue mimicking CFS [27].

Jacobson et al. [28] assayed serum folate levels in 60 patients with CFS and reported a high prevalence of severe folate deficiency even though they did not use more sensitive methods for detection of folate deficiency such as formiminoglutamic acid excretion test or deoxyuridine suppression test [28, 29]. Folate metabolism plays a central role in human lymphocyte development and function [30–33]. It is therefore plausible that the inappropriate antibody responses of CFS patients are in part owing to an underlying folate deficiency.

A high incidence of severe B cell immunodeficiency and chronic reactivated EBV infection in patients with CFS is reported herein. Also reported is that a significant portion of these patients benefit from treatment with folinic acid (CAS 58-05-9), which shows that CFS frequently is a folinic acid responsive clinical entity accompanied by B cell immunodeficiency and inappropriate antibody responses to EBV.

## 2. Patients and methods

### 2.1. CFS Patients

Fifty-eight (58) patients with CFS were evaluated. Patient data are summarized in Table 1. Most patients (90 %) were women. The patients ranged in age from 19 to 64 years (median, 45 years). Twenty-six patients (45 %) had CFS or CFS plus fibromyalgia (FM) without other major co-morbidities. The co-morbidities of the remainder included rheumatoid arthritis (RA) (22 %), systemic lupus erythematosus (SLE) (10 %), inflammatory bowel disease (IBD) (16 %), and multiple sclerosis (MS)

**Table 1: Patient characteristics and folinic acid response.**

| | | | |
|---|---|---|---|
| Female | 52/58 | | 90 % |
| Age (years): | | median: | 45 |
| | | range: | 19–64 |
| CFS or CFS + FM | | | 26/58 (45 %) |
| CFS + RA | | | 13/58 (22 %) |
| CFS + SLE | | | 6/58 (10 %) |
| CFS + IBD | | | 9/58 (16 %) |
| CFS + MS | | | 4/58 (7 %) |
| Previous EBV exposure | | | 58/58 (100 %) |
| Reactivated EBV infection | | | 42/58 (72 %) |
| T-cell immunodeficiency[a)] | | | 9/35 (26 %) |
| B-cell immunodeficiency[b)] | | | 33/35 (94 %) |

[a)] % CD3+ < 50 % of MNC.
[b)] % CD19+ sIgM+ < 10 % of MNC.

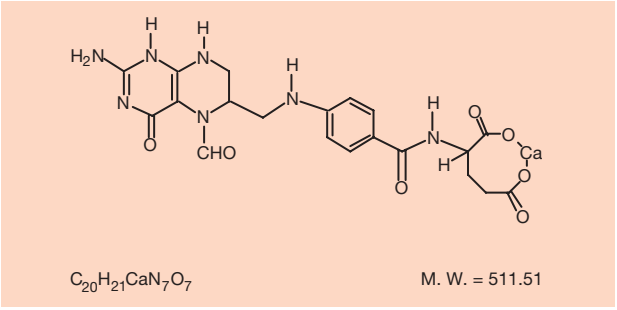


**Fig. 1: Structural formula of folinic acid, also known as Citrovorum factor, or 5-formyl-5,6,7,8-tetrahydrofolic acid; chemical designation: calcium N-[p-[[(6RS)-2-amino-5-formyl-5,6,7,8-tetrahydro-4-hydroxy-6-pteridinyl] methyl] amino] benzoyl]-L-glutamate (1:1).**

(7 %) (Table 1). Clinical and laboratory information was collected and used for retrospective analysis according to the guidelines of the Parker Hughes Institute Institutional Review Board (IRB) for secondary use of existing data.

### 2.2. Laboratory tests

Immunophenotyping of Ficoll-Hypaque separated peripheral blood mononuclear cells from CFS patients was performed using multiparameter flow cytometry using a broad panel of monoclonal antibodies reactive with surface lymphocyte differentiation antigens, as previously described in detail [34, 35]. The standard EBV serology testing was performed by LabCorp (Kansas City, USA) and included testing of serum specimens from CFS patients for IgG and IgM antibodies against EBV VCA, EA, and NA.

### 2.3. Folinic acid

Folinic acid (Fig. 1) is a mixture of the diastereoisomers of the 5-formyl derivative of tetrahydrofolic acid. The biologically active compound in the mixture is the (–)-/- isomer, known as Citrovorum factor. Folinic acid does not require reduction by the enzyme dihydro folate reductase in order to participate in reactions utilizing folates as a source of one carbon moieties. 5-Formyl tetrahydrofolate (synonyms: leucovorin, Citrovorum factor) was purchased from Cardinal Health (Hudson, WI, USA). Folinic acid is a derivative of tetrahydrofolic acid, the reduced form of folic acid, which is involved as a cofactor for 1-carbon transfer reactions in the biosynthesis of purines and pyrimidines of nucleic acids. Normal total serum folate concentrations range from 5 to 15 ng/mL. Normal cellular folate concentrations range from 175 to 315 ng/mL. Following oral administration of a 15 mg dose, mean peak serum folate concentrations of 268 ng/mL occur within 1.7 h. In vivo folinic acid is converted to other tetrahydrofolic acid derivatives, including 5-methyl tetrahydrofolate, which is the major transport and storage form of folate in the body. The terminal half-life for total reduced folates is 6.2 h. Folinic acid was used at a 25 mg dose level 3–4 times daily for 1–2 months and patients were interviewed to determine the effects of folinic acid on their fatigue and pain.

## 3. Results

### 3.1. Reactivated EBV infection in patients with CFS

Fifty-eight patients with CFS were evaluated for prior EBV exposure using serologic testing for anti-EBV antibodies and all 58 (100 %) had evidence of prior EBV exposure, as documented by the presence of EBV VCA IgG in their serum whereas 42 patients (72 %) had evidence for reactivated EBV infection, as documented by the presence of EBV EA IgG in their serum (Table 1, Table 2). Of these 42 patients, 3 (7 %) also had IgM antibodies to EBV VCA in addition to IgG antibodies to EBV VCA. Nine (21 %) CFS patients with VCA IgM-negative, EA IgG-positive reactivated EBV infection had either no detectable or very low levels of NA IgG, consistent with an inappropriate B cell immune response to EBV (Table 2). In addition, 6 patients with VCA IgG positivity had undetectable or very low levels of NA IgG, consistent with either an inappropriate EBV antibody response, chronic reactivated EBV infection, or both (Table 2).

### 3.2. Depletion of mature B-lymphocyte pool in patients with CFS

Peripheral blood mononuclear cells from 35 of the 58 CFS patients were immunophenotyped. The majority of the mononuclear cells were T lymphocytes expressing the T cell differentiation antigens (Table 3). The mean percent positivity for CD2, CD3, CD4, CD5, and CD7 antigens were 59 ± 3 %, 54 ± 3 %, 51 ± 3 %, 54 ± 3 %, and 57 ± 3 %, respectively (Table 3). B-lineage lymphoid cells expressing CD19, CD20, or surface IgM represented a small minority of the mononuclear cells (Table 3). Nine patients (26 %) had T-cell immunodeficiency with less than 50 % (actual percentages: 11 %, 13 %, 28 %, 28 %, 31 %, 32 %, 32 %, 40 %) CD3+ mature T-lymphocytes (Table 1). Notably, 33 patients (94 %) had B-cell immunodeficiency with less than 10 % CD19+IgM+ mature B-lymphocytes (Table 1).

### 3.3. Folinic acid responsiveness of fatigue and pain in patients with CFS

Because of the previously reported association between folate deficiency and CFS, the observed quantitative

**Table 2: EBV serology data.**

| | |
|---|---|
| VCA antibodies: | |
| $IgM^+$ | 3/58 (5 %) |
| $IgG^+$ | 58/58 (100 %) |
| EA antibodies: | |
| $IgG^+$ | 42/58 (72 %) |
| NA antibodies: | |
| $IgG^{-/low}$ | 15/58 (26 %) |
| $IgG^+$ | 43/58 (74 %) |
| VCA $IgM^+$, VCA $IgG^+$, NA $IgG^+$, EA $IgG^+$ | 3/58 (5 %) |
| VCA $IgM^-$, VCA $IgG^+$, NA $IgG^{-/low}$, EA $IgG^+$ | 9/58 (16 %) |
| VCA $IgM^-$, VCA $IgG^+$, NA $IgG^+$, EA $IgG^+$ | 30/58 (52 %) |
| VCA $IgM^-$, VCA $IgG^+$, NA $IgG^{-/low}$, EA $IgG^-$ | 6/58 (10 %) |
| VCA $IgM^-$, VCA $IgG^+$, NA $IgG^+$, EA $IgG^-$ | 10/58 (17 %) |

**Table 3: Immunophenotypic features of peripheral blood mononuclear cells for patients with CFS.**

| | | Percent positive |
|---|---|---|
| T-cell markers: | | |
| CD2 | mean ± SE | 59 ± 3 |
| | median (range) | 62 (8–81) |
| CD3 | mean ± SE | 54 ± 3 |
| | median (range) | 57 (11–80) |
| CD4 | mean ± SE | 51 ± 3 |
| | median (range) | 56 (16–81) |
| CD5 | mean ± SE | 54 ± 3 |
| | median (range) | 59 (10–80) |
| CD7 | mean ± SE | 57 ± 3 |
| | median (range) | 60 (6–83) |
| B-cell markers: | | |
| CD19 | mean ± SE | 10 ± 1 |
| | median (range) | 8 (1–47) |
| CD20 | mean ± SE | 16 ± 2 |
| | median (range) | 14 (4–53) |
| B-cell populations: | | |
| $CD19^+$ $CD20^+$ | % of lymphoid cells | |
| | mean ± SE | 11 ± 2 |
| | median (range) | 9 (2–48) |
| | % of total MNC | |
| | mean ± SE | 8 ± 1 |
| | median (range) | 8 (1–31) |
| $CD19^+$ $sIgM^+$ | % of lymphoid cells | |
| (Mature B) | mean ± SE | 9 ± 2 |
| | median (range) | 7 (0.5–53) |

**Table 4: Folinic acid responsiveness of CFS.**

| Patient population | Improved after folinic acid |
|---|---|
| All patients (N = 42) | 34 (81 %) |
| CFS or CFS + FM (N = 20) | 14 (70 %) |
| CFS + IBD (N = 8) | 6 (75 %) |
| CFS + RA (N = 8) | 8 (100 %) |
| CFS + SLE (N = 5) | 5 (100 %) |
| CFS + MS (N = 1) | 1 (–) |
| EA $IgG^-$ (N = 13) | 10 (77 %) |
| EA $IgG^+$ (N = 29) | 24 (83 %) |

and functional deficits in B cell immunity of CFS patients prompted us to empirically treat 42 CFS patients with oral folinic acid. Folic acid deficiency was not examined using serum folate measurements since there is no available standardized test for folic acid deficiency in CNS [36] and no information is available about the normal CSF folate levels in healthy volunteers. Folinic acid was chosen because of its proven efficacy in the treatment of congenital as well as acquired folate deficiency, including CNS folate deficiency. Thirty-four patients (81 %) reported significant subjective improvement with increased energy level and reduced pain within 2 months (Table 1). No patient reported side effects from folinic acid. Responses were documented in all CFS patients regardless of their comorbidities or EBV EA antibody status (Table 4). Several patients also reported improvement in the numbness of their hands and feet but no formal evaluation of their presumed sensory neuropathy was performed before and after folinic acid (Lundell, unpublished observations).

## 4. Discussion

The cause of CFS remains to be deciphered through epidemiological research [37]. Patients with CFS have been reported to display various immunologic abnormalities [38]. A number of studies suggested a state of generalized immune activation and selective immune dysfunction in patients with CFS. For example, hyperactivition of an unwanted cellular cascade by the immune-related protein RNase L has been linked to reduced exercise capacity in persons with CFS [39]. Robertson et al. reported an abundance of immature CD16+CD3- natural killer cells in CFS patients [40].

It has been suggested that CFS may be associated with chronic active infection due to the Epstein-Barr virus. Analysis of reports to date shows that the mean titers of antibodies to VCA and to early antigen EA are greater for patients with CFS than for healthy individuals and it has also been reported that patients with CFS have greater numbers of EBV-infected lympocytes than control subjects [41]. Unlike acute infectious mononucleosis, wherein EBV establishes lifelong infection and survives by maintaining a delicate balance with the host as a latent infection, in chronic active EBV infection the host-virus balance is disturbed [42]. There is growing evidence that the serologic findings of an enhanced EBV state in CFS patients reflect a generalized underlying immunologic dysfunction in these patients [43]. A subset of CFS patients was reported to have IgM antibodies to EBV [44].

Intriguingly, EBV deoxyuridine triphosphate nucleotidohydrolase (dUTPase), an early nonstructural EBV-encoded protein, has been shown to cause immune dysregulation and produce clinical symptoms observed in patients with CFS separate from its role in virus replication and may serve as a new approach to help identify one of the etiological agents for CFS [45].

It has also been suggested that a hypofunctional hypothalamic-pituitary-adrenal axis in chronic fatigue syndrome could result in an exaggerated release of pro-inflammatory cytokines during stress. Since pro-inflammatory cytokines are involved in the induction of sickness behavior and thus constitute a potential physiological correlate of stress-induced symptom exacerbation in chronic fatigue syndrome, Gaab et al. recently evaluated the lipopolysaccharide (LPS)-induced production of pro-inflammatory cytokines during psychosocial stress in CFS and healthy controls. CFS patients did not show an exaggerated secretion of LPS-induced cytokines. Although cortisol responses to stress were normal, pro-inflammatory cytokine levels in CFS patients were significantly attenuated [46].

A high incidence of severe B cell immunodeficiency and chronic reactivated EBV infection in patients with CFS is reported herein. Also reported is that a significant portion of these patients benefit from treatment with folinic acid, which supports the notion that CFS frequently is a folic acid responsive clinical entity accompanied by B cell immunodeficiency and inappro-

priate antibody responses to EBV. Folinic acid was previously shown to be an effective agent for treatment of patients with a deficiency of dihydropteridine reductase (DHPR) and presumed CNS folate deficiency[47]. It is also effective in treatment of severe neuropathy and fatigue caused by the chemotherapeutic drugs vincristine [48], vinblastine [49], and methotrexate [50, 51]. The observed activity of folinic acid in patients with CFS may provide the foundation for a new standard of care for this frequently debilitating condition.

## Literature


[1] Reid, S., Chalder, T., Cleare, A. et al., Chronic fatigue syndrome. Br. Med. J. **320**, 292 (2000)

[2] Joyce, J., Hotopf, M., Wessely, S., The prognosis of chronic fatigue and chronic fatigue syndrome: a systematic review. QJM **90**, 223 (1997)

[3] Strauss, S., Chronic Fatigue Syndrome, in: Braunwald, E., Fauci, A. S., Kasper, D. L. et al. (eds.), Harrison's Principles of Internal Medicine, 15th ed., pp. 2541–2542. Mc Graw-Hill, New York (2001)

[4] Kim, E., A brief history of chronic fatigue syndrome. JAMA **272**, 1070 (1994)

[5] Chronic fatigue syndrome. Clinical practice guidelines. Med. J. Aust. **176** (Suppl.), S23 (2002)

[6] Schluederberg, A., Straus, S. E., Peterson, P. et al., NIH conference. Chronic fatigue syndrome research. Definition and medical outcome assessment. Ann. Intern. Med. **117**, 325 (1992)

[7] Goldenberg, D. L., Simms, R. W., Geiger, A. et al., High frequency of fibromyalgia in patients with chronic fatigue seen in a primary care practice. Arthritis Rheum. **33**, 381 (1990)

[8] Aaron, L. A., Burke, M. M., Buchwald, D., Overlapping conditions among patients with chronic fatigue syndrome, fibromyalgia, and temporomandibular disorder. Arch. Intern. Med. **160**, 221 (2000)

[9] Kroenke, K., Wood, D. R., Mangelsdorff, A. D. et al., Chronic fatigue in primary care. Prevalence, patient characteristics, and outcome. JAMA **260**, 929 (1988)

[10] Wessely, S., Chalder, T., Hirsch, S. et al., The prevalence and morbidity of chronic fatigue and chronic fatigue syndrome: a prospective primary care study. Am. J. Public Health **87**, 1449 (1997)

[11] Fukuda, K., Straus, S. E., Hickie, I. et al., The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. Ann. Intern. Med. **121**, 953 (1994)

[12] Vollmer-Conna, U., Hickie, I., Hadzi-Pavlovic, D. et al., Intravenous immunoglobulin is ineffective in the treatment of patients with chronic fatigue syndrome. Am. J. Med. **103**, 38 (1997)

[13] Straus, S. E., Dale, J. K., Tobi, M. et al., Acyclovir treatment of the chronic fatigue syndrome. Lack of efficacy in a placebo-controlled trial. N. Engl. J. Med. **319**, 1692 (1988)

[14] Whiting, P., Bagnall, A. M., Sowden, A. J. et al., Interventions for the treatment and management of chronic fatigue syndrome: a systematic review. JAMA **286**, 1360 (2001)

[15] Straus, S. E., Pharmacotherapy of chronic fatigue syndrome: another gallant attempt. JAMA **292**, 1234 (2004)

[16] Gilliam, A. Epidemiologic study of an epidemic diagnosed as poliomyelitis, occurring among personnel of Los Angeles County General Hospital during the summer of 1934: Bull no 240. Washington DC, US Public Health Service, Division of Infectious Diseases, National Institutes of Health, Bethesda, MD, USA (1938)

[17] An outbreak of encephalomyelitis in the Royal Free Hospital Group, London, in 1955. Br. Med. J. **33**, 895 (1957)

[18] Levine, P. H., Dale, J. K., Benson-Grigg, E. et al., A cluster of cases of chronic fatigue and chronic fatigue syndrome: clinical and immunologic studies. Clin. Infect. Dis. **23**, 408 (1996)

[19] Holmes, G. P., Kaplan, J. E., Stewart, J. A. et al., A cluster of patients with a chronic mononucleosis-like syndrome. Is Epstein-Barr virus the cause? JAMA **257**, 2297 (1987)

[20] Mawle, A. C., Nisenbaum, R., Dobbins, J. G. et al., Seroepidemiology of chronic fatigue syndrome: a case-control study. Clin. Infect. Dis. **21**, 1386 (1995)

[21] Acheson, E. D., The clinical syndrome variously called benign myalgic encephalomyelitis, Iceland disease and epidemic neuromyasthenia. Am. J. Med. **26**, 569 (1959)

[22] Henderson, D. A., Shelokov, A., Epidemic neuromyasthenia; clinical syndrome. N. Engl. J. Med. **260**, 757 (1959)

[23] Jones, J. F., Ray, C. G., Minnich, L. L. et al., Evidence for active Epstein-Barr virus infection in patients with persistent, unexplained illnesses: elevated anti-early antigen antibodies. Ann. Intern. Med. **102**, 1 (1985)

[24] Straus, S. E., Tosato, G., Armstrong, G. et al., Persisting illness and fatigue in adults with evidence of Epstein-Barr virus infection. Ann. Intern. Med. **102**, 7 (1985)

[25] Straus, S. E., The chronic mononucleosis syndrome. J. Infect. Dis. **157**, 405 (1988)

[26] Georgiades, E., Behan, W. M., Kilduff, L. P. et al., Chronic fatigue syndrome: new evidence for a central fatigue disorder. Clin. Sci. (Lond). **105**, 213 (2003)

[27] Servaes, P., Prins, J., Verhagen, S. et al., Fatigue after breast cancer and in chronic fatigue syndrome: similarities and differences. J. Psychosom. Res. **52**, 453 (2002)

[28] Jacobson, W., Saich, T., Borysiewicz, L. K. et al., Serum folate and chronic fatigue syndrome. Neurology **43**, 2645 (1993)

[29] Gimsing, P., Melgaard, B., Andersen, K. et al., Vitamin B-12 and folate function in chronic alcoholic men with peripheral neuropathy and encephalopathy. J. Nutr. **119**, 416 (1989)

[30] Ames, B. N., Cancer prevention and diet: help from single nucleotide polymorphisms. Proc. Natl. Acad. Sci. USA **96**, 12216 (1999)

[31] Wang, X., Thomas, P., Xue, J. et al., Folate deficiency induces aneuploidy in human lymphocytes in vitro-evidence using cytokinesis-blocked cells and probes specific for chromosomes 17 and 21. Mutat. Res. **551**, 167 (2004)

[32] Krajinovic, M., Lamothe, S., Labuda, D. et al., Role of MTHFR genetic polymorphisms in the susceptibility to childhood acute lymphoblastic leukemia. Blood **103**, 252 (2004)

[33] Lightfoot, T. J., Roman, E., Causes of childhood leukaemia and lymphoma. Toxicol. Appl. Pharmacol. **199**, 104 (2004)

[34] Uckun, F. M., Gaynon, P., Sather, H. et al., Clinical features and treatment outcome of children with biphenotypic CD2+ CD19+ acute lymphoblastic leukemia: a Children's Cancer Group study. Blood **89**, 2488 (1997)

[35] Uckun, F. M., Ledbetter, J. A., Immunobiologic differences between normal and leukemic human B-cell precursors. Proc. Natl. Acad. Sci. USA **85**, 8603 (1988)

[36] Snow, C. F., Laboratory diagnosis of vitamin B12 and folate deficiency: a guide for the primary care physician. Arch. Intern. Med. **159**, 1289 (1999)

[37] Ranjith, G., Epidemiology of chronic fatigue syndrome. Occup. Med. (Lond). **55**, 13 (2005)

[38] Glaser, R., Kiecolt-Glaser, J. K., Stress-associated immune modulation: relevance to viral infections and chronic fatigue syndrome. Am. J. Med. **105**, 35S (1998)

[39] Snell, C. R., Vanness, J. M., Strayer, D. R. et al., Exercise capacity and immune function in male and female patients with chronic fatigue syndrome (CFS). In Vivo **19**, 387 (2005)

[40] Robertson, M. J., Schacterle, R. S., Mackin, G. A. et al., Lymphocyte subset differences in patients with chronic fatigue syndrome, multiple sclerosis and major depression. Clin. Exp. Immunol. **141**, 326 (2005)

[41] Jones, J. F., Serologic and immunologic responses in chronic fatigue syndrome with emphasis on the Epstein-Barr virus. Rev. Infect. Dis. **13** (Suppl. 1), S26 (1991)

[42] Maia, D. M., Peace-Brewer, A. L., Chronic, active Epstein-Barr virus infection. Curr Opin Hematol. **7**, 59 (2000)

[43] Sumaya, C. V., Serologic and virologic epidemiology of Epstein-Barr virus: relevance to chronic fatigue syndrome. Rev. Infect. Dis. **13** (Suppl. 1), S19 (1991)

[44] Lerner, A. M., Beqaj, S. H., Deeter, R. G. et al., IgM serum antibodies to Epstein-Barr virus are uniquely present in a subset of patients with the chronic fatigue syndrome. In Vivo **18**, 101 (2004)

[45] Glaser, R., Padgett, D. A., Litsky, M. L. et al., Stress-associated changes in the steady-state expression of latent Epstein-Barr virus: implications for chronic fatigue syndrome and cancer. Brain Behav. Immun. **19**, 91 (2005)

[46] Gaab, J., Rohleder, N., Heitz, V. et al., Stress-induced changes in LPS-induced pro-inflammatory cytokine production in chronic fatigue syndrome. Psychoneuroendocrinology **30**, 188 (2005)

[47] Woody, R. C., Brewster, M. A., Glasier, C., Progressive intracranial calcification in dihydropteridine reductase deficiency prior to folinic acid therapy. Neurology **39**, 673 (1989)

[48] Grush, O. C., Morgan, S. K., Folinic acid rescue for vincristine toxicity. Clin. Toxicol. **14**, 71 (1979)

[49] Conter, V., Rabbone, M. L., Jankovic, M. et al., Overdose of vinblastine in a child with Langerhans' cell histiocytosis: toxicity and salvage therapy. Pediatr. Hematol. Oncol. **8**, 165 (1991)

[50] Riva, L., Conter, V., Rizzari, C. et al., Successful treatment of intrathecal methotrexate overdose with folinic acid rescue: a case report. Acta Paediatr. **88**, 780 (1999)

[51] Shuper, A., Stark, B., Kornreich, L. et al., Methotrexate-related neurotoxicity in the treatment of childhood acute lymphoblastic leukemia. Isr. Med. Assoc. J. **4**, 1050 (2002)